RESEARCH

Effect of non-protein factors on heat stability of Chilean Sauvignon Blanc wines

Johannes de Bruijn[1*], Cristina Loyola[1], José Luis Arumí[1], and José Martínez[2]

Protein denaturation in white wines results into a hazy suspension with precipitate formation, affecting negatively their commercialization. The aim of this study was to identify a number of non-protein factors of wines, which exhibit a modulating effect upon haze formation and interfere with protein precipitation in white wines, applying stepwise multiple regression analysis. The influence of intrinsic non-protein factors, including surface and groundwater quality, on protein haze formation assessed by the heat test was studied. Experiments were performed on 18 Sauvignon Blanc wines from six Chilean valleys. The influence of non-protein factors (pH, electrical conductivity [EC], total and volatile acidity, alcohol, free and total sulfur dioxide, total polyphenols, and chloride, sulfate, K, Na, Ca, Mg, and Fe concentrations) on haze response was evaluated by means of multiple regression analysis. Significant contribution ($p < 0.05$) of EC, sulfate and Fe concentrations to protein haze was found. Due to multi-collinearity between sulfate and Fe concentrations, the multi-linear model of haze response was reduced to: Haze = -184 + 2.95 × [Protein] - 62.3 × [Fe] + 0.17 × EC ($r_a = 0.901$). Electric conductivities of wine and surface water were correlated ($p = 0.037$); good correlations were also found between sulfate concentrations in wine and surface water ($p = 0.003$), and groundwater ($p = 0.022$). No correlation was detected for Fe. This study elucidates that protein haze formation in white wine is a multi-factorial process. Iron, EC, and sulfate, in addition to protein itself, have to be considered as factors that modulate wine protein hazing.

Key words: Electrical conductivity, haze, iron, multiple regression analysis, sulfate, *Vitis vinifera*.

INTRODUCTION

It is widely accepted that proteins belong to one of the most important compounds in white wines that affect quality by contributing to their sensorial and foam characteristics (Dambrouck et al., 2005; Cilindre et al., 2007). However, white wines without bentonite fining exhibit normally turbidity formation when exposed to increasing temperatures (30 to 80 °C) followed by storage at 4 °C (Mesquita et al., 2001). Specific wine proteins, identified mainly as pathogenesis-related proteins, are heat unstable, resulting into haze formation (Waters et al., 1996; Waters et al., 1998). Protein instability in wines is a two-step phenomenon: protein unfolding, a temperature protein denaturation results into a hazy suspension with precipitate formation, which is a considerable drawback in bottled wines -mediated step, followed by colloidal aggregation due to intermolecular interactions among unfolded proteins (Waters et al., 2005; Dufrechou et al., 2010).

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Although protein type is the primary factor controlling the nature and concentration of haze particles, the exact mechanism responsible for protein haze formation in white wines is still not fully understood. Several authors have suggested that some non-protein components (specific anions, phenol compounds, organic acids, and polysaccharides), as well as pH and ionic strength modulated protein aggregation (Mesquita et al., 2001; Pocock et al., 2007; Batista et al., 2010; Marangon et al., 2011; Dufrechou et al., 2012; Gazzola et al., 2012). However, the interactions between all these different factors still need to be investigated to understand their precise role in protein haze formation in wines.

It has been suggested that the profiles of proteins and non-protein components of white wines would be affected in a complex manner by factors such as grapevine (*Vitis vinifera* L.) cultivar, viticulture, and wine making practices, soil, climate and vintage year. In particular, grapevine cultivar and growing region were factors with a significant influence on the protein profile of a given wine, the former being particularly predominant. On the other hand, the influence of wine making practice seems to be a negligible factor for the protein profiles of wines (Sarmento et al., 2001). Additionally, the mineral element profiles of wines are often utilized as a fingerprint for their characterization and the zoning of geographical regions (Saavedra et al., 2011). These chemical elements are considered to be good indicators for zoning because they are not metabolized or modified during vinification.

However, final concentrations of mineral elements may vary with time and wine making methods.

One of the most important export products of Chile is wine. Chilean wine industry has grown drastically over the last two decades, where total wine production increased 2.44-fold from 1990 to 2009 reaching 10.1 million hL (OIV, 2014). Chilean wines show a wide diversity and are generally of high quality due to Chilean geography with a lot of different terroir and zones for grapevine growing. Fourteen wine valleys can be distinguished starting at the Elqui valley in the North (29.9° S lat) to the Malleco valley in the South (38° S lat) with approximately 900 km between them, affected by a marine climate of the Pacific Ocean at the west coast and the Andes mountains with volcanic activity at the east side. The vineyards in the Central valley receive their water resources by superficial and groundwater fluxes going from the Andes in direction to the Pacific Ocean. Because of the wide diversity of landscape, Chile may be considered as a unique natural laboratory to study the influence of geological, climatologic, and hydrological phenomena by means of chemical speciation into the formation of commercially unacceptable haze or deposits during the storage of bottled wines.

The aim of this study was to identify a number of non-protein factors of wines, which exhibit a modulating effect upon haze formation and interfere with protein precipitation in white wines, applying stepwise multiple regression analysis. In addition, some abiotic factors which may affect grapevine quality, such as hydrological, geological, and climatic aspects were included to assess their influence on the non-protein factors mentioned before for Sauvignon Blanc coming from six Chilean wine valleys using a Geographic Information System (GIS).

MATERIALS AND METHODS

Wine samples

Samples of Sauvignon Blanc, vintage 2010, were obtained from six commercial vineyards of an industrial winery located in different valleys of the central region, Chile (Table 1). Three samples were taken at random from different wine tanks at each site. All wines came from the same vine clone (clone 1). Vines without water deficit were between 4 and 10 yr old at the start of the experiment and received the same agronomic management. Wines were made using the same protocol (De Bruijn et al., 2009). After visual inspection for the presence of moulds, fruit was de-stemmed and crushed, followed by cooling of the pulp to about 10 °C, sulfur dioxide (SO_2) addition, and maceration by using pectinase. Then pulp was pressed, particles settled down over night and the must filtered, followed by alcoholic fermentation. After about 6 wk, the wine was racked and the SO_2 level was adjusted to prevent oxidation and malolactic fermentation. Wine samples were transported at about 15 °C to the Pilot Plant of the Department of Agroindustry, settled for 40 h at 4 °C, centrifuged (SAT 130, Bertuzzi, Milan, Italy) at 1440 × *g* and filtered (Whatman nr 42, Whatman, Maidstone, UK) with a precoat (4.07 kg m^{-2}) of diatomaceous earth nr 13 (Furet, Chillán, Chile). Then wines were stored in glass jars (1 L) in the dark at 4 °C prior to chemical analysis.

Table 1. Sources of Sauvignon Blanc samples from six valleys of the central region, Chile.

Notation	Vineyard location	Valley
LI	30°31' S, 71°28' W	Limarí
CA	33°20' S, 71°25' W	Casablanca
MO	33°39' S, 70°34' W	Maipo
RC	34°03' S, 71°40' W	Rapel Costa
CO	35°03' S, 71°16' W	Curicó
ME	35°32' S, 71°29' W	Maule

Analytical methods

Protein haze was determined by the heat test according to Moine-Ledoux and Dubourdieu (1999) with some modifications. Each wine sample (25 mL) was transferred into a test tube, sealed with a screw cap and heated at 80 °C for 2 h in a thermostat-controlled water bath, then held at 4 °C for 2 h, and warmed at room temperature for 2 h. Turbidity was measured before and after heat treatment using the Hach 2100P turbidimeter (Hach, Loveland, Colorado, USA), where turbidity was expressed in nephelometric turbidity units (NTU). Protein haze was expressed as the difference of wine turbidity before and after heat treatment. Total soluble protein concentration of wine samples was determined according to the Bradford method (Bradford, 1976), where absorbance was measured at 595 nm, using bovine serum albumin (Calbiochem, La Jolla, California, USA) as a standard.

Volumetric degree of alcohol, chloride, sulfate, total and volatile acidity, pH, free and total SO_2, and electrical conductivity (EC) were measured according to the official methods of analysis of CEE (CEE, 1990). Total polyphenols were measured by the Folin-Ciocalteu assay, using the Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) and reading absorbance at 750 nm with the Sunny UV-7804C spectrophotometer (Sunny Optical Technology, Yuyao, Zhejiang, China) (Singleton and Rossi, 1965). Concentrations were expressed as equivalents of gallic acid (Sigma-Aldrich, St. Louis, Missouri, USA). Total concentrations of K, Na, Ca, Mg, and Fe were measured by flame atomic absorption spectrometry (FAAS) on a Unicam Solaar 969, FAA spectrometer (Thermo Scientific [TJA Solutions], Waltham, Massachusetts, USA). All analyses were carried out in triplicate. Values obtained from the wine analyses were within the legal intervals established by the European Union (Tables 2a and 2b).

Statistical analysis

Bivariate regression analysis was used to assess the relationship between the haze formation in wine and

Table 2a. Physical and chemical properties of Sauvignon Blanc wines.

Property	Sample								
	LI-1	LI-2	LI-3	CA-1	CA-2	CA-3	MO-1	MO-2	MO-3
Haze, NTU	68 ± 0	69 ± 1	69 ± 1	101 ± 17	83 ± 16	117 ± 17	128 ± 1	130 ± 1	131 ± 2
Protein, mg L^{-1}	15 ± 3	18 ± 4	11 ± 3	22 ± 2	19 ± 1	21 ± 1	24 ± 0	25 ± 1	25 ± 0
Alcohol, % vol	13.3 ± 0.1	13.7 ± 0.1	13.7 ± 0.1	13.7 ± 0.1	13.9 ± 0.1	13.9 ± 0.1	12.3 ± 0.1	11.8 ± 0.1	12.3 ± 0.1
Chloride, g L^{-1}	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.01	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.01	0.15 ± 0.01	0.18 ± 0.02
Sulfate, g L^{-1}	0.84 ± 0.03	0.79 ± 0.03	0.81 ± 0.02	0.99 ± 0.04	0.91 ± 0.02	0.91 ± 0.02	1.26 ± 0.04	1.16 ± 0.05	1.15 ± 0.04
Total acidity, g L^{-1}	6.9 ± 0.0	7.0 ± 0.1	7.1 ± 0.1	7.1 ± 0.0	7.2 ± 0.1	7.2 ± 0.1	6.8 ± 0.0	6.9 ± 0.0	6.8 ± 0.0
Volatile acidity, g L^{-1}	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
pH	3.31 ± 0.01	3.31 ± 0.00	3.30 ± 0.01	3.39 ± 0.00	3.40 ± 0.01	3.40 ± 0.01	3.43 ± 0.02	3.42 ± 0.02	3.40 ± 0.01
SO_2 free, mg L^{-1}	15 ± 1	15 ± 1	17 ± 0	12 ± 0	12 ± 1	11 ± 0	10 ± 0	10 ± 0	10 ± 0
SO_2 total, mg L^{-1}	100 ± 1	100 ± 1	99 ± 0	64 ± 1	64 ± 1	63 ± 0	55 ± 1	54 ± 1	54 ± 0
EC, S m^{-1}	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.16 ± 0.00	0.16 ± 0.00	0.16 ± 0.00
Polyphenols, mg L^{-1}	254 ± 9	258 ± 9	272 ± 7	254 ± 18	290 ± 9	273 ± 18	236 ± 4	235 ± 1	260 ± 2
K, mg L^{-1}	606 ± 7	622 ± 8	612 ± 3	672 ± 4	668 ± 2	676 ± 4	764 ± 13	782 ± 10	790 ± 15
Na, mg L^{-1}	12 ± 1	11 ± 0	16 ± 1	13 ± 1	9 ± 1	8 ± 0	8 ± 0	8 ± 0	7 ± 0
Ca, mg L^{-1}	49 ± 3	58 ± 2	55 ± 4	43 ± 1	44 ± 1	45 ± 1	53 ± 1	54 ± 1	55 ± 1
Mg, mg L^{-1}	90 ± 1	92 ± 1	90 ± 0	89 ± 2	85 ± 1	88 ± 2	82 ± 2	80 ± 1	77 ± 2
Fe, mg L^{-1}	0.32 ± 0.02	0.40 ± 0.02	0.30 ± 0.02	0.28 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.54 ± 0.02	0.47 ± 0.02	0.45 ± 0.02

LI: Limarí, CA: Casablanca, MO: Maipo, NTU: nephelometric turbidity units.

Table 2b. Physical and chemical properties of Sauvignon Blanc wines.

Property	Sample								
	RC-1	RC-2	RC-3	CO-1	CO-2	CO-3	ME-1	ME-2	ME-3
Haze, NTU	63 ± 5	51 ± 5	58 ± 6	70 ± 7	63 ± 8	56 ± 6	49 ± 1	47 ± 1	47 ± 0
Protein, mg L^{-1}	13 ± 3	21 ± 5	17 ± 3	20 ± 3	16 ± 1	17 ± 1	15 ± 4	9 ± 2	17 ± 4
Alcohol, % vol	13.6 ± 0.1	13.5 ± 0.0	13.5 ± 0.0	13.3 ± 0.1	13.3 ± 0.0	13.1 ± 0.0	13.0 ± 0.0	13.0 ± 0.0	13.1 ± 0.0
Chloride, g L^{-1}	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.12 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	0.26 ± 0.00	0.26 ± 0.00	0.26 ± 0.00
Sulfate, g L^{-1}	0.23 ± 0.01	0.27 ± 0.01	0.24 ± 0.01	0.24 ± 0.02	0.23 ± 0.01	0.19 ± 0.00	1.04 ± 0.03	0.95 ± 0.04	0.97 ± 0.03
Total acidity, g L^{-1}	6.7 ± 0.0	6.7 ± 0.0	6.7 ± 0.0	7.4 ± 0.0	7.4 ± 0.0	7.4 ± 0.0	7.4 ± 0.0	7.5 ± 0.0	7.4 ± 0.0
Volatile acidity, g L^{-1}	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
pH	3.45 ± 0.01	3.45 ± 0.01	3.44 ± 0.00	3.35 ± 0.02	3.36 ± 0.02	3.40 ± 0.01	3.36 ± 0.01	3.33 ± 0.01	3.34 ± 0.01
SO_2 free, mg L^{-1}	6 ± 0	6 ± 0	6 ± 0	18 ± 1	18 ± 1	17 ± 0	13 ± 0	14 ± 1	14 ± 1
SO_2 total, mg L^{-1}	49 ± 1	51 ± 2	49 ± 1	118 ± 2	123 ± 3	123 ± 3	69 ± 1	69 ± 1	72 ± 2
EC, S m^{-1}	0.13 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00
Polyphenols, mg L^{-1}	258 ± 3	260 ± 9	265 ± 4	226 ± 8	235 ± 9	241 ± 7	203 ± 9	258 ± 7	234 ± 9
K, mg L^{-1}	692 ± 9	684 ± 18	658 ± 9	732 ± 4	738 ± 2	730 ± 3	692 ± 8	702 ± 9	712 ± 9
Na, mg L^{-1}	15 ± 1	17 ± 1	14 ± 0	7 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0
Ca, mg L^{-1}	37 ± 3	37 ± 1	48 ± 2	71 ± 2	72 ± 1	75 ± 1	71 ± 1	73 ± 1	72 ± 1
Mg, mg L^{-1}	78 ± 3	82 ± 3	87 ± 3	71 ± 2	68 ± 2	72 ± 2	90 ± 1	90 ± 1	88 ± 1
Fe, mg L^{-1}	0.65 ± 0.01	0.72 ± 0.02	0.68 ± 0.01	0.87 ± 0.01	0.80 ± 0.01	0.83 ± 0.01	0.45 ± 0.02	0.36 ± 0.01	0.43 ± 0.02

RC: Rapel Costa, CO: Curicó, ME: Maule, NTU: nephelometric turbidity units.

its protein concentration by means of the Pearson's correlation coefficient (r). Data were analyzed by one-way ANOVA and data significance was assessed by the Student's t-test at $p < 0.05$. To get additional evidence on the influence of intrinsic non-protein factors and protein concentration on haze formation, a stepwise multiple regression analysis for a multi-linear model was applied. The response was described by:

$$Haze = \beta_0 + \beta_1 f_1 + \sum_{i=2}^{n} \beta_I f_I$$

where β_I and f_I are the coefficient and the concentration of proteins, respectively; β_i is the coefficient and f_i is the value of the non-protein factors; $_0$ a lumping coefficient. The non-protein factors that have been considered were medium characteristics (pH, EC, total and volatile acidity), uncharged compounds (alcohol, total polyphenols, free and total SO_2), anions (chloride and sulfate) and cations (K, Na, Ca, Mg, and Fe) of the original wine samples. The inclusion of these factors in the model was determined by stepwise regression at 95% confidence level using the Statgraphics Centurion XVI.I software (Statistical Graphics Corp., Warrenton, Virginia, USA). This regression involved the t-test to differentiate which non-protein factor is relevant to haze formation, eliminating in each step a coefficient (β_i) that was not significantly different from zero, until all remaining coefficients were significant. To compare multi-linear models with different number of independent variables, adjusted multiple correlation coefficients (r_a) were calculated and compared with the correlation coefficient of the bivariate regression model. The adjusted multiple correlation coefficients were used as a criterion to distinguish between relevant and non relevant non-protein factors from the haze point of view. Moreover, Durbin-Watson (DW) statistic was used to test multi-collinearity or autocorrelation between independent variables. Then, regression analysis was used to assess

the correlation between the concentration of some non-protein compounds and characteristics of wine samples, and surface water (DGA, 1996) or groundwater (SISS, 2014) by means of the Pearson's correlation coefficient (r). Significance of data was assessed bilaterally by the t-test at $p = 0.05$ and $p = 0.01$, using the SPSS software, version 15.0 (SPSS, Chicago, Illinois, USA). Finally, the Arcgis 10.1 software (Esri, Redlands, California, USA) was used for mapping and geographical analysis of the geochemical and geophysical data of winery locations, surroundings and valleys, by combining the vector GIS and raster GIS models.

RESULTS AND DISCUSSION

Variables affecting protein haze formation

In order to evaluate the correlation between total protein concentration and haze formation in Sauvignon Blanc wines, a significant and positive correlation between these variables was found ($p < 0.05$; $r = 0.78$) after using bivariate regression analysis. Although present in rather small amounts, proteins are of primary importance for colloidal stability and clarity of white wines. However, several protein fractions showed different sensitivity to heat denaturation (Hsu and Heatherbell, 1987; Waters et al., 1991; Sauvage et al., 2010). In particular, chitinases and thaumatin-like proteins, which constitute the major part of white wine proteins, are important contributors to heat-induced protein instability (Waters et al., 1992; Dawes et al., 1994).

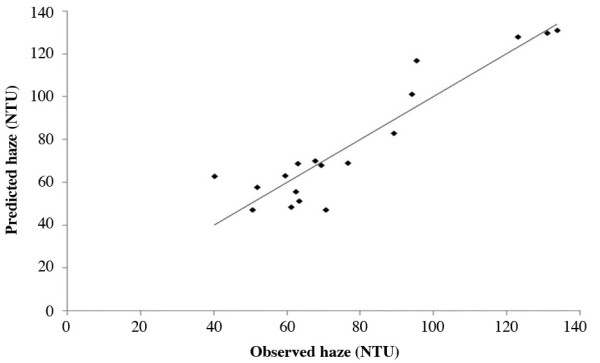
There is a lot of evidence that wine components other than proteins are absolutely required for the formation of protein haze in white wines. Wine instability may be influenced by many non-protein factors, including wine pH (Mesquita et al., 2001; Batista et al., 2009), ionic strength (Marangon et al., 2011), ethanol content (Sarmento et al., 2000), concentrations of polysaccharides (Waters et al., 1993; Mesquita et al., 2001), polyphenols (Waters et al., 1995; Pocock et al., 2007; Esteruelas et al., 2011), organic acids (Batista et al., 2010), and sulfate (Pocock et al., 2007; Marangon et al., 2011). Applying stepwise multiple regression analysis to our data of chemical composition and attributes of Sauvignon Blanc, a significant contribution ($p < 0.05$) of EC, sulfate and Fe concentrations to protein haze was found with adjusted multiple correlation coefficients higher than the correlation coefficient between protein concentration and haze formation (Table 3). After the incorporation of at least two non-proteins factors, the complexity of the linear model was increased, but the correlation was improved according to the higher values of r_a (Table 3). However, multi-collinearity between the concentrations of sulfate and Fe was detected. Therefore, sulfate and Fe concentrations should not be used at the same time as independent variables in a model used to predict protein haze formation. Highest adjusted multiple correlation coefficient ($r_a = 0.910$) was found using protein and Fe concentrations, and EC as independent variables in our model, where Haze = $-184 + 2.95 \times$ [Protein] $- 62.3 \times$ [Fe] $+ 0.17 \times$ EC (Table 3). The plot of the observed values *vs*. predicted values using the above model for protein haze formation showed a fairly good fit ($R^2 = 0.858$) (Figure 1). Increasing protein concentrations in the range between 9 and 25 mg L^{-1} and EC between 0.134 and 0.163 S m^{-1} results into more visible haze formation, while low Fe concentrations (between 0.3 and 0.9 mg L^{-1}) seem to improve protein stability of white wines.

Electrical conductivity of wine is related to its ionic strength by means of the concentration of electrolyte species. Conductivity depends upon the kind of electrolytes, where conductivity is directly proportional to their concentration. On the other hand, ionic strength is a

Table 3. Coefficients (β_i) of the multi-linear model of haze response, adjusted multiple correlation coefficients (r_a) and Durbin-Watson p-values for multi-collinearity testing of protein and non-protein factors in wine samples.

Variable(s)	β_0	β_1	β_2	β_3	r_a	Durbin-Watson p-value
Protein	-13.7	5.10			0.775	
Protein – pH	141.4	5.35	-47.3		0.743	0.235
Protein – EC	-170.6	3.28	0.13		**0.807**	0.153
Protein – Total acidity	92.8	4.82	-14.4		0.751	0.323
Protein – Volatile acidity	-23.5	4.75	34.8		0.747	0.337
Protein – Alcohol	115.7	4.62	-9.14		0.760	0.349
Protein – Free SO_2	-13.0	5.09	-0.043		0.740	0.312
Protein – Total SO_2	2.85	4.83	-0.15		0.753	0.333
Protein - Total polyphenols	-83.6	5.17	0.27		0.766	0.440
Protein - Chloride	-4.45	4.95	-41.5		0.743	0.388
Protein - Sulfate	-22.3	4.35	0.030		**0.837**	0.066
Protein - K	-38.7	4.84	0.043		0.743	0.367
Protein - Na	-9.60	5.07	-0.36		0.742	0.284
Protein - Ca	6.05	4.86	-0.27		0.750	0.398
Protein - Mg	-57.1	5.32	0.47		0.750	0.136
Protein - Fe	9.87	5.23	-51.2		**0.825**	0.087
Protein – Sulfate – Fe	-12.4	4.59	0.022	-16.8	**0.827**	**0.035**
Protein – Sulfate – EC	-141	3.07	0.026	0.10	**0.860**	0.215
Protein – Fe - EC	-184	2.95	-62.3	0.17	**0.910**	0.170

EC: Electrical conductivity; ra: adjusted multiple correlation coefficient.



NTU: Nephelometric turbidity units.

Figure 1. Plot of the observed (measured) haze versus the haze predicted by the model equation with the independent variables of electrical conductivity, protein, and Fe concentrations.

function of the molality and the charge of ionic species in the wine, where ions with higher valence show a stronger contribution to ionic strength. In particular, the interaction of wine proteins with other charged wine constituents, such as polysaccharides and organic acids, has shown to be strongly dependent on their net charges and on the nature of their functional groups, where electrostatic and ionic forces are determinant affecting wine stability (Vernhet et al., 1996; Batista et al., 2010). The aggregation for a given protein is considered to be a two-step mechanism, where structural changes (unfolding) are followed by colloidal interactions (Dufrechou et al., 2012). Colloidal aggregation of macromolecules results from a complex interplay between usually attractive van der Waals and hydrogen-bonding interactions and repulsive electrostatic interactions between species carrying the same charge. Increasing ionic strength will decrease the ionic double-layer thickness, reducing electrostatic interactions, thus favoring the aggregation of proteins (Marangon et al., 2011). Thus ionic strength may indeed be one of the additional factors to be required for protein haze formation in white wines.

Sulfate in our wines was present in a concentration range varying from 189 up to 1262 mg L^{-1}, with a mean of 732 mg L^{-1}. Pocock et al. (2007) suggest that sulfate was required for protein haze formation in white wines. Sulfate mediated the aggregation of unfolded chitinase, but not of thaumatin-like proteins, indicating a different behavior of these proteins towards this bivalent ion (Marangon et al., 2011). Protein self-aggregation is likely to take place being facilitated by the screening of protein charges by sulfate anions.

In this study Fe concentrations of wines varied between 0.3 and 0.9 mg L^{-1}, with a mean of 0.5 mg L^{-1}. Iron is usually present in commercial white wines at a concentration range varying from of 1.2 and 3.8 mg L^{-1} (McKinnon and Scollary, 1997; Riganakos and Veltsistas, 2003). Our data suggest that Fe at low concentrations is an essential factor to improve protein stability in white wines. Proteins rich in cystein are able to chelate metal ions. These proteins are generally heat unstable having between 14 and 16 cysteins per molecule, while a heat stable protein such as vacuolar grape invertase contains only five cystein groups per molecule (De Bruijn et al., 2013). In particular ferric ions may oxidize cystein groups located at the protein, which may result into additional intramolecular disulfide bonds, stabilizing the protein structure and avoiding the interaction with other macromolecules. Therefore, a minor haze potential might be expected for Fe species at trace levels.

Environmental influence

After computing the Pearson's correlation coefficients between surface or groundwater and wine data, a significant positive correlation ($p < 0.05$) was found for sulfate and Mg concentrations (Tables 4 and 5); EC was also positively correlated ($p < 0.05$) in case of surface water and wine (Table 5). Sulfate concentration and EC of water resources used to irrigate grapevines seem to be important parameters for protein hazing of white wines. On the other hand, pH, Fe, chloride, Ca, Na, and K concentrations did not show any significant correlation for both kinds of water sources and wine.

Table 4. Pearson's correlation (significance) between chemical elements in surface water and white wine.

	EC water	Fe water	SO_4 water	Cl water	Ca water	Mg water	Na water	K water
EC	0.494*							
wine	(0.037)							
Fe		-0.130						
wine		(0.607)						
SO_4			0.658**					
wine			(0.003)					
Cl				-0.336				
wine				(0.172)				
Ca					-0.224			
wine					(0.372)			
Mg						0.487*		
wine						(0.041)		
Na							0.177	
wine							(0.483)	
K								0.165
wine								(0.512)

*Correlation is significant at a level of 0.05 (bilateral).
**Correlation is significant at a level of 0.01 (bilateral).

Table 5. Pearson's correlation (significance) between chemical elements in groundwater and white wine.

	pH water	Fe water	SO_4 water	Cl water	Mg water
pH	0.286				
wine	(0.249)				
Fe		0.120			
wine		(0.635)			
SO_4			0.534**		
wine			(0.022)		
Cl				-0.257	
wine				(0.304)	
Mg					0.497*
wine					(0.036)

*Correlation is significant at a level of 0.05 (bilateral).

The content of minerals in wines can be attributed to several natural sources, such as atmospheric deposition of airborne particulate matter on grapes, and the transfer of these species from soil and water resources via the roots to the grapes, and to contamination during winemaking, residues of agrochemical products used during grapevine growing and other anthropogenic activities. Anions, such as sulfate and chloride, are normally available as free species in the soil solution, because soil particles contain predominantly fixed negative charges and act as cation exchanger. Sulfate in the soil comes predominantly from the weathering of parent rock material according to Epstein and Bloom (2005). On the other hand, the relatively low sulfate concentrations measured in wines from the Rapel Costa (RC) valley (246 mg L^{-1}) in contrast to the Maipo (MO) valley (1191 mg L^{-1}) suggests that marine deposition of salts is of minor importance compared to other atmospheric depositions such as the volcanic activity of San José de Maipo and the contamination from the city of Santiago de Chile. In addition, highest concentrations of chloride (0.263 g L^{-1}) were found in this study for the Maule (ME) valley at 100 km from the coast with a geology that was predominated by volcanic deposition of fine ash in contrast to the Limarí (LI), RC, and Casablanca (CA) valleys nearby the Pacific Ocean with a mean concentration of chloride of 0.133 g L^{-1} in the wines. Several authors reported that plants may acquire considerable amounts of sulfate and chloride from the atmosphere and not just in locations nearby the ocean (Pessarakli, 1995; Epstein and Bloom, 2005). Moreover, the presence of Mg in surface and groundwater sources is due to the lixiviation from minerals (pyroxenes) and should interact with negatively charged soil particles.

The highest values of EC were measured for wines from the MO and Curicó (CO) valleys having 0.161 and 0.147 S m^{-1}, respectively. A relatively far distance (90-100 km) to the coast yields more extreme temperatures. High temperatures result into increased evaporation of water from grapevines, yielding higher mineral concentrations in the grapes. More water uptake by the plants is required to maintain adequate water potential, whereby the mineral composition of irrigation water may affect protein hazing of wines. According to our knowledge, no systematic studies have been reported for a relationship between soil chemistry, irrigation of grapevines growing in semi-arid Chilean valleys and wine quality. This study suggests that vineyards of white grapevines should be situated nearby some water source (sea, lake, or river) to avoid water evaporation from grapes in excess in order to evade high salt concentrations, which increase protein hazing potential of white wines.

Mineral composition (Fe, chloride, Ca, Na, and K) of vine grapes does normally not reflect that of the surroundings media (groundwater and surface water). Species such as Fe, though present in relative abundance in the soil solution, are present only in trace amounts in the grapevine and finally in the wine. The plasma membrane serves as a barrier to the uncontrolled entry of solutes, where specific carriers on the cell membranes allow the absorption of specific ions of importance to the organism. The lowest Fe concentrations were detected in Sauvignon wines from CA and LI valleys with relatively high pH values (7.67 and 7.42, respectively) for the groundwater. On the other hand, a reduced availability of Fe has been reported for plants growing on alkaline and calcareous soils with low biological activity (Pessarakli, 1995; Epstein and Bloom, 2005). Low release of CO_2 from roots, and enzymes and organic acids from microorganisms will not contribute to the acidification of soil, diminishing the desorption rate of Fe from the solid phase into the soil solution.

CONCLUSIONS

This study confirms that protein haze formation in white wines is a multi-factorial process. Iron, electrical conductivity and sulfate, in addition to protein itself, should be considered as factors that modulate the wine protein hazing potential. Mineral composition (iron and salt concentrations) of wines is related to the geographical region in which the grapes were grown due to its relationship with soil composition and climate differences. Finally, mineral composition (sulfate and salt concentrations) of wines can be deduced from the composition of irrigation water.

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